PATENT
IN THE UNITED STATED PATENT AND TRADEMARK OFFICE

#5

RE	:	Office Letter dated March 6th, 2001
TITLE	:	METHODS FOR MAKING AND DELIVERING RHO-ANTAGONIST TISSUE ADHESIVE FORMULATIONS TO THE INJURED MAMMALIAN CENTRAL AND PERIPHERAL NERVOUS SYSTEMS AND USES THEREOF
APPLICANT/INVENTOR	:	MCKERRACHER, Lisa
FILED	:	November 30th, 2000
SERIAL NO.	:	09/725,906
GROUP ART UNIT	:	1615
ATTORNEY DOCKET NO:		06447-003-US-02

Montréal, Québec, Canada
August 2, 2001

RESPONSE

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

The present is in response to the Office Letter dated March 6, 2001. The response was due on May 6, 2001. The applicant by separate letter requests an extension of time up to and including August 6, 2001.

Accordingly, please amend the above-identified US Patent Application as follows:

IN THE DISCLOSURE:

In the disclosure, please delete the following passage found on pages 41 to 45;

[(see identification of Rho antagonist section).

SEQUENCE of (known) Rho antagonist C3 used in the experiments

Nucleotide sequence including part of the plasmid GST sequence. The vector with the GST sequence is commercially available and thus the entire GST sequence including the start was not sequenced. It was desired to determine only the sequence 3 ' to the thrombin cleavage site which releases C3 from the GST sequence. The thrombine cleavage site is shown with an arrow and is located just to the left of the underlined nucleotide sequence below (i.e. the arrow shows the thrombin cleavage site). The underlined sequence shows additional coding sequence translated in our recombinant protein that is not reported in the literature.

Both strands were sequenced to verify that there were no errors in the sequence.

↓

5' GTG GCG ACC CTT CCC AAA TCG GAT CTG GTT CCG CGT GGA TCC TCT AGA
GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC
CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT
GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA
CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA
TTT AGA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA
AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA TTT GCA GGA AGA
CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT
AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC
GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
GCT ATC AAT CCT AAA TAA 3'

Nucleotide sequence of recombinant C3 protein: the sequence given below represents the entire coding sequence for the Rho antagonist used in the experments mentioned herein. It is similar to the sequence shown above but does not include the GST portion which when the protein is made is enzymatically removed with thrombin.

1 GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA
51 AAAGGCTTAT TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA
101 AAGCTTGGGG TAATGCTCAG TATAAAAAGT ATGGACTAAG CAAATCAGAA
151 AAAGAAGCTA TAGTATCATA TACTAAAAGC GCTAGTGAAA TAAATGGAAA
201 GCTAAGACAA AATAAGGGAG TTATCAATGG ATTTCCTTCA AATTTAATAA
251 AACAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAAATGAA GACCCCTGAA
301 AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT

351 TCAAAACACT CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG
401 AAAAGGCTAA AGCTAAGTTT TTAAATAAAG ATAGACTTGA ATATGGATAT
451 ATTAGTACTT CATTAATGAA TGTTTCTCAA TTTGCAGGAA GACCAATTAT
501 TACAAAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT ATTGACCCTA
551 TTAGTGCTTT TCAGGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT
601 TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT
651 TACAGCAACA ATGATGGGCA CAGCTATCAA TCCTAAATAA

Amino acid sequence (one letter code)

Translation of the above sequence to show amino acid sequence. Amino acids in bold, highlight differences from published sequence (Popoff et al. (1990) Nucl. Acid. Ress. 18:1291. EMBL accession no. X511464.) The 11 N-terminal sequences are additional; there is a single amino acid change of an alanine (hydrophobic) to glutamic acid (Q).

GSSRVDLQAC NAYSINQKAY SNTYQEFTNI DQAKAWGNAQ YKKYGLSKSE KEAIVSYTKS ASEINGKLRQ NKGVINGFPS NLIKQVELLD KSFNKMKTPE NIMLFXGDDP AYLGTEFQNT LLNSNGTINK TAFEKAKAKF LNXDRLEYGY ISTSLMNVSQ FAGRPIITKF KVAKGSKAGY IDPISAF**Q**GQ LEMLLPRHST YHIDDMRLSS DGKQIIITAT MMGTAINPK

Number of amino acids: 229

Molecular weight: 25507.5

Theoretical pI: 9.43

Amino acid composition:

Ala (A) 18 7.9%
Arg (R) 6 2.6%
Asn (N) 18 7.9%
Asp (D) 10 4.4%
Cys (C) 1 0.4%
Gln (Q) 12 5.2%
Glu (E) 10 4.4%
Gly (G) 16 7.0%
His (H) 2 0.9%
Ile (I) 18 7.9%

Leu (L) 17 7.4%
Lys (K) 23 10.0%
Met (M) 7 3.1%
Phe (F) 10 4.4%
Pro (P) 7 3.1%
Ser (S) 20 8.7%
Thr (T) 14 6.1%
Trp (W) 1 0.4%
Tyr (Y) 11 4.8%
Val (V) 6 2.6%

Asx (B) 0 0.0%
Glx (Z) 0 0.0%
Xaa (X) 2 0.9%

Total number of negatively charged residues (Asp + Glu): 20
Total number of positively charged residues (Arg + Lys): 29

Estimated half-life:

The N-terminal of the sequence considered is G (Gly).

The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).
>20 hours (yeast, in vivo).
>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 26.88
This classifies the protein as stable.

Aliphatic index: 75.07
Grand average of hydropathicity (GRAVY): -0.479]

Please also delete the following passage found on pages 46 to 51;

[SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: LISA MCKERRACHER

(ii) TITLE OF INVENTION: Methods for making and delivering Rho-antangonist tissue adhesive formulations to the injured mammalian central and peripheral nervous systems and uses thereof

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:
(A) ADRESSEE: BROULLETTE KOSIE
(B) STREET: 1100 RENE-LESVEQUE BLVD WEST
(C) PROV/STATE: QUEBEC
(D) COUNTRY: CANADA
(E) POSTAL/ZIP CODE: H3B 5C9

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE:ASCII (TEXT)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) ATTORNEY/AGENT INFORMATION:
(A) NAME: RONALD S. KOSIE
(B) REGISTRATION NO.: 28,814
(C) REFERENCE/DOCKET NO.: 06447-003-US-2
(D) TEL. NO.: (514) 397 8500
(E) FAX NO.: (514) 397 8515

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:

(B) TYPE:

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GTG GCG ACC CTT CCC AAA TCG GAT CTG GTT CCG CGT GGA TCC TCT AGA

5 10 15

GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT

20 25 30

TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG

35 40 45

GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA

50 55 60

GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA

65 70 75 80

AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA

85 90 95

CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA

100 105 110

AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA

115 120 125

TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT

130 135 140

TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT AGA CTT GAA TAT

145 150 155 160

GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA TTT GCA GGA AGA

165 170 175

CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT

180 185 190

ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT

195 200 205

AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT

210 215 220

AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT

225 230 235 240

AAA TAA

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:

(B) TYPE:

(C) STRANDEDNESS:

(D) TOPOLOGY:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(ix) FEATURE:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA 50
AAAGGCTTAT TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA 100
AAGCTTGGGG TAATGCTCAG TATAAAAAGT ATGGACTAAG CAAATCAGAA 150
AAAGAAGCTA TAGTATCATA TACTAAAAGC GCTAGTGAAA TAAATGGAAA 200
GCTAAGACAA AATAAGGGAG TTATCAATGG ATTTCCTTCA AATTTAATAA 250
AACAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAAATGAA GACCCCTGAA 300
AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT 350
TCAAAACACT CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG 400
AAAAGGCTAA AGCTAAGTTT TTAAATAAAG ATAGACTTGA ATATGGATAT 450
ATTAGTACTT CATTAATGAA TGTTTCTCAA TTTGCAGGAA GACCAATTAT 500
TACAAAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT ATTGACCCTA 550
TTAGTGCTTT TCAGGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT 600
TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT 650
TACAGCAACA ATGATGGGCA CAGCTATCAA TCCTAAATAA

(2) INFORMATION FOR SEQ ID NO: 3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:
(B) TYPE:
(C) STRANDEDNESS:
(D) TOPOLOGY:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(ix) FEATURE:
(D) OTHER INFORMATION:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GSSRVDLQAC NAYSINQKAY SNTYQEFTNI DQAKAWGNAQ YKKYGLSKSE 50
KEAIVSYTKS ASEINGKLRQ NKGVINGFPS NLIKQVELLD KSFNKMKTPE 100

NIMLFXGDDP AYLGTEFQNT LLNSNGTINK TAFEKAKAKF LNXDRLEYGY 150
ISTSLMNVSQ FAGRPIITKF KVAKGSKAGY IDPISAFQGQ LEMLLPRHST 200
YHIDDMRLSS DGKQIIITAT MMGTAINPK]

IN THE DRAWINGS:

Please replace the drawings presently on file (i.e. figures 1A, 1B, 2, 3, 4a, 4b, 4c, 4d, 5a, 5b, 5c, 6A, 6B, 7A, 7b, 7C, 8 and to 9) with the new formal drawings (i.e. figures 1A, 1B, 2, 3, 4A, 4B, 4C, 4D, 5A, 5B, 5C, 6A, 6B, 7A, 7B, 7C, 8 and 9) which are submitted herewith.

REMARKS:

By the present amendment, the applicant wishes to delete from the disclosure the sequence listings referred to on pages 41 to 45, and 46 to 51.

In light of the above, the applicant hereby respectfully requests that the separate sequence listings be withdrawn.

By the present the applicant has further included substitute drawings in compliance with 37 CFR 1.84. The applicant also wishes hereby to make editorial amendments to the drawings. These amendments are highlighted in red in photocopies of the drawings originally submitted. As you will notice, these amendments generally refers to titles of the figures. In order to facilitate matters, formal drawings have been included in the present response.

As requested in the outstanding Office Letter of March 6, 2001, the applicant hereby includes the declaration for patent application and appointment of attorney. The US Patent Office is hereby authorized to charge the amount of $130.00 required for late declaration to our **Deposit Account no. 02-3980** .

As mentioned above, the applicant has by separate letter petitioned for a three (3) month extension of time within which to respond to the outstanding Office Letter of March 6, 2001,

namely up to and including August 6, 2001. If any further extension of time is necessary, the United States Patent and Trademark Office is hereby petitioned for such an extension and may charge any necessary fees to our **Deposit Account no. 02-3980.**

If any further fee, **whatsoever**, with respect to the present application is due, the United States Patent and Trademark Office is in any event hereby authorized to charge such further amount to our **Deposit Account no. 02-3980**.

Favourable consideration of the present application in light of the foregoing amendments and remarks is respectfully requested.

Respectfully submitted,

BROUILLETTE KOSIE

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Reg. No. 28,814
1100 René-Lévesque Blvd West
25th Floor
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Canada H3B 5C9

Telephone: (514) 397-6942
Fax: (514) 397-8515

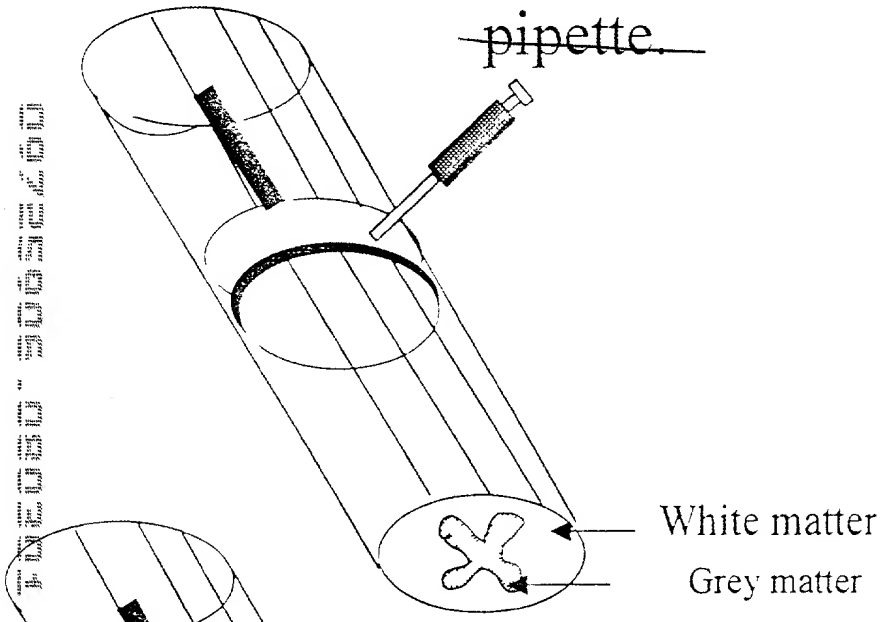
(Docket no. 06447-003-US-02)

Encl. Petition for extension of time
Initial drawings (i.e. figures 1A, 1B, 2, 3, 4a, 4b, 4c, 4d, 5a, 5b, 5c, 6A, 6B, 7A, 7b, 7C, 8 and to 9) with amendments outlined generally in red
New formal drawings (i.e. figures 1A, 1B, 2, 3, 4A, 4B, 4C, 4D, 5A, 5B, 5C, 6A, 6B, 7A, 7B, 7C, 8 and 9)
Declaration for patent application and appointment of attorney
Copy of USPTO notice
Confirmation receipt post card

Figure 1

Delivery of Rho-antagonist tissue adhesive formulation.

1A. Application of tissue adhesive + Rho antagonist to the injured spinal cord with a pipette.



1B. Axon regeneration through the adhesion matrix + C3

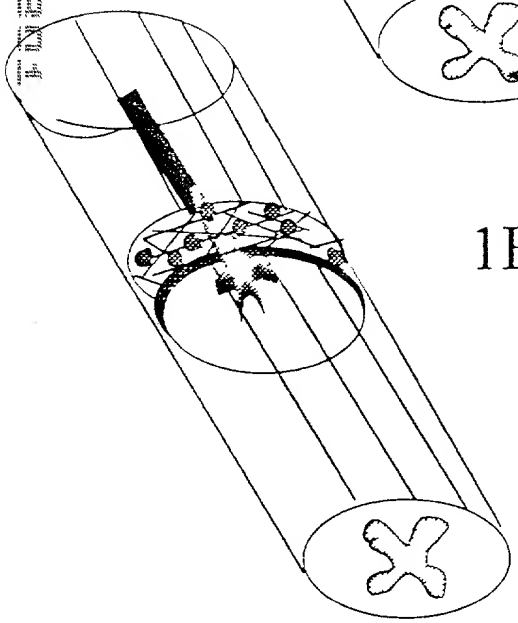


Figure 2

~~Lesion of Corticospinal tract~~

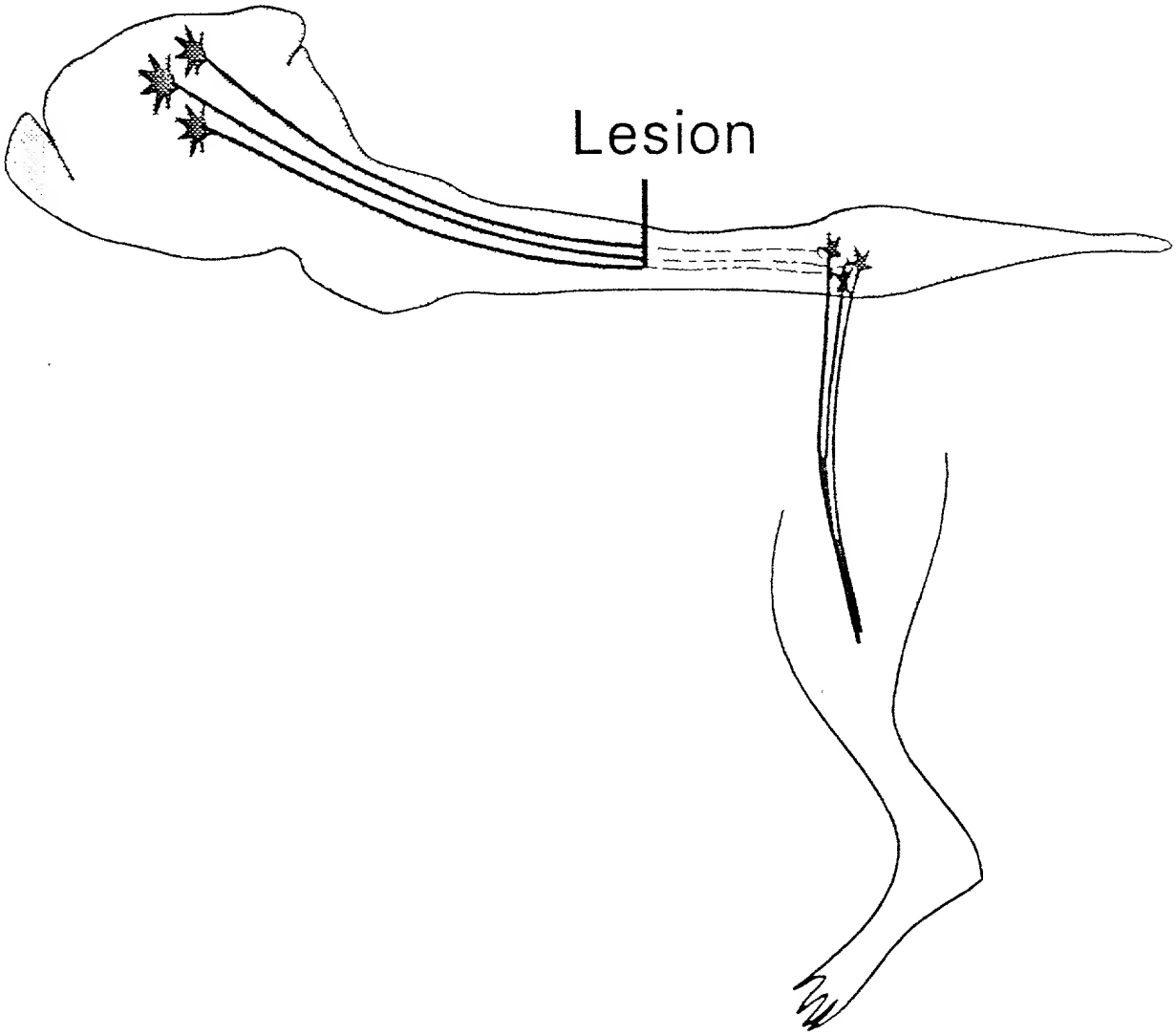
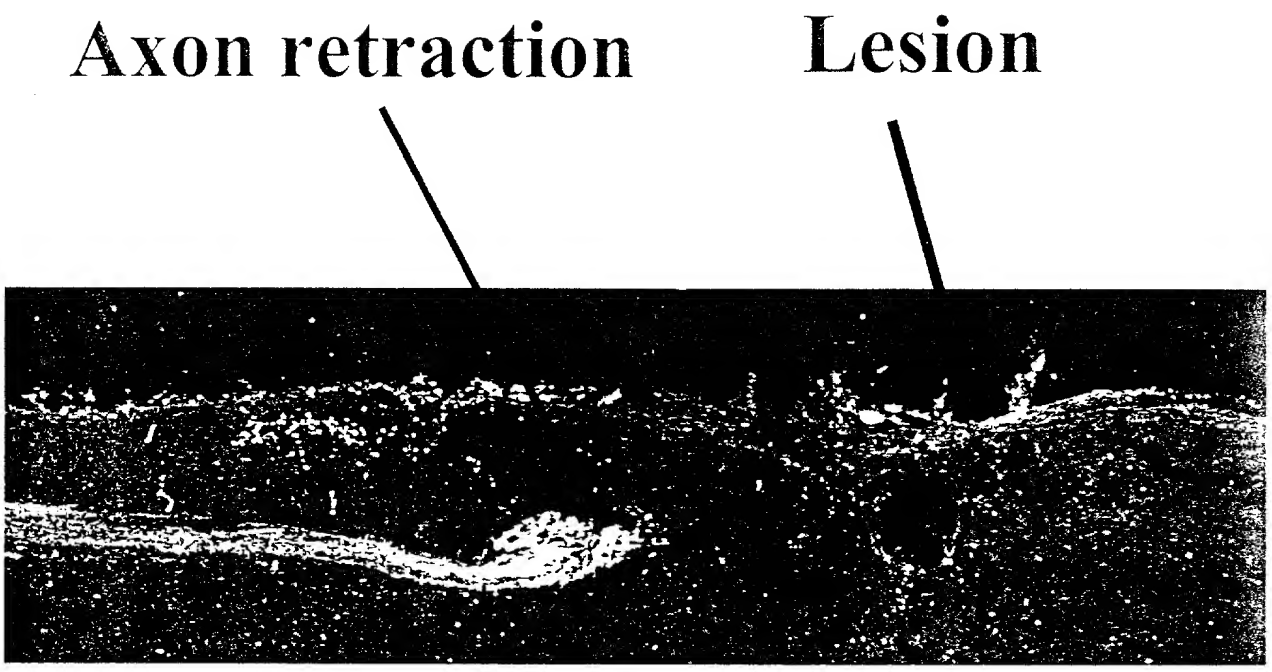


Figure 3

~~Corticospinal tract lesion (untreated adult mice)~~



~~Figure 4~~

Figure 4a

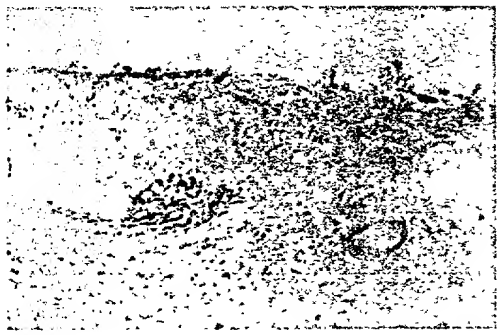


Figure 4b

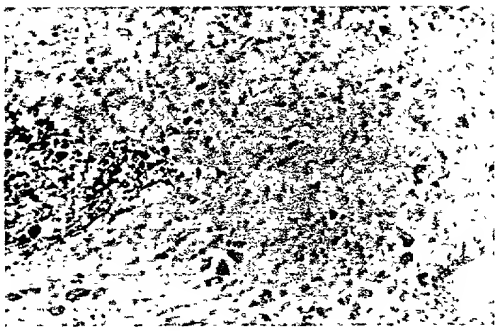


Figure 4c

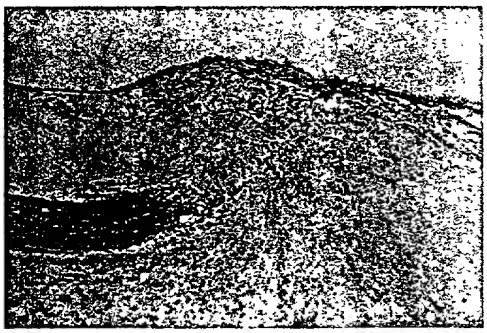


Figure 4d

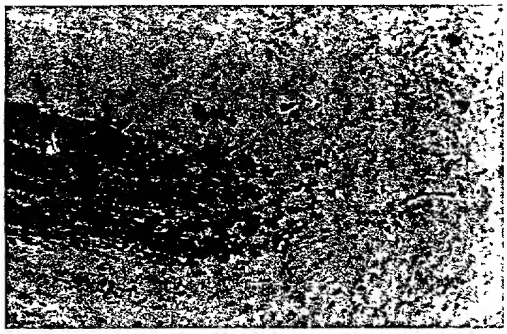


Figure 5: **Effect of C3/fibrin treatment on injured corticospinal tract**

Figure 5a

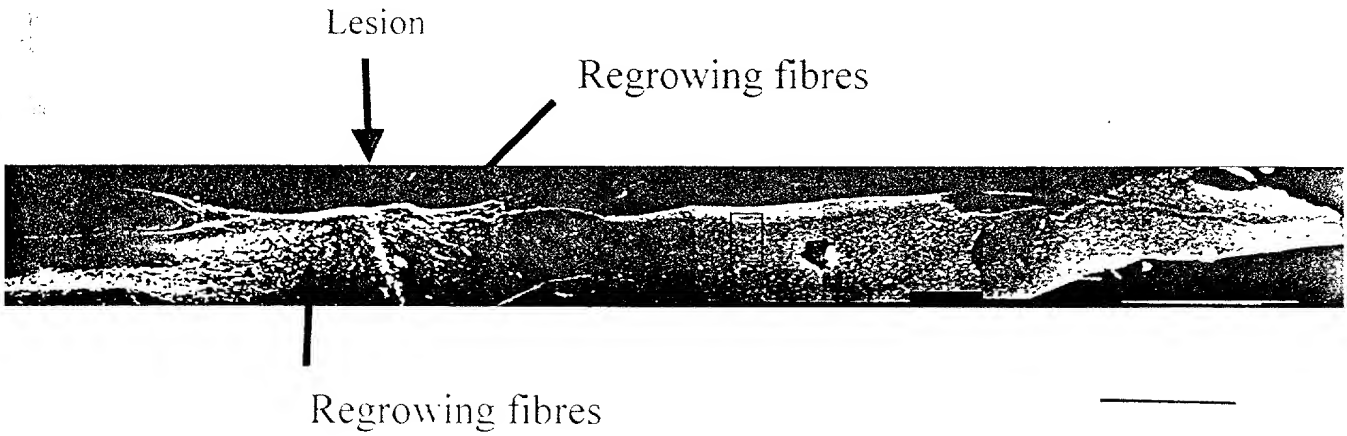


Figure 5b

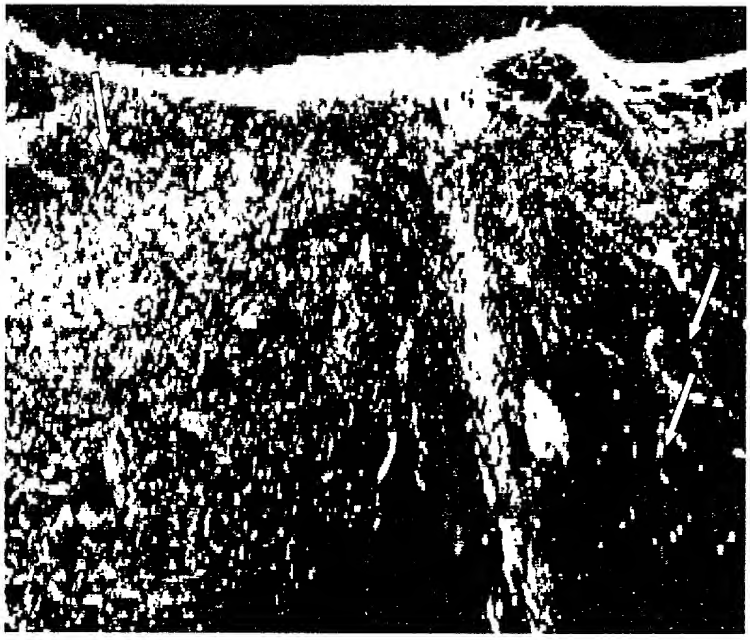


Figure 5c

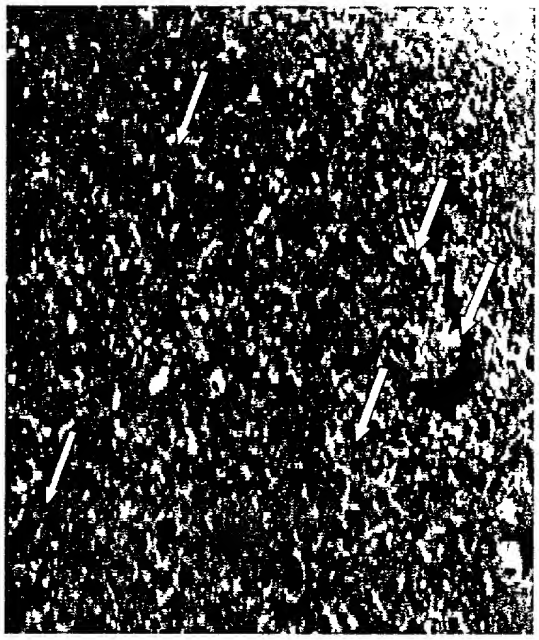


Figure 6: C3/fibrin glue -treated spinal cord

Figure 6A

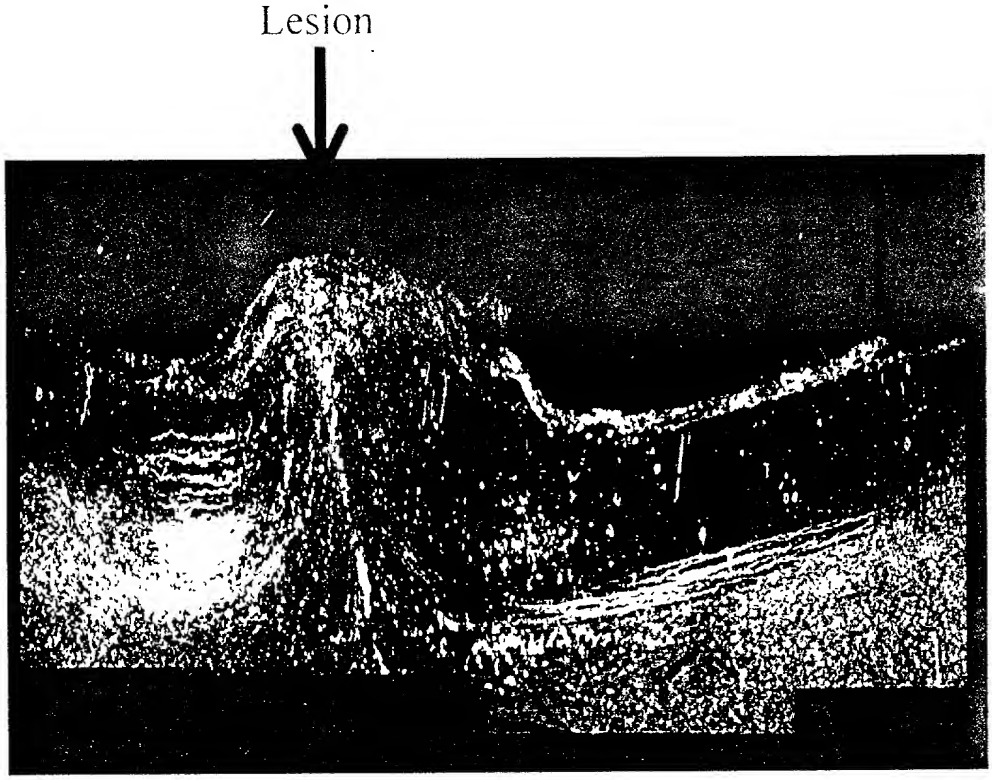
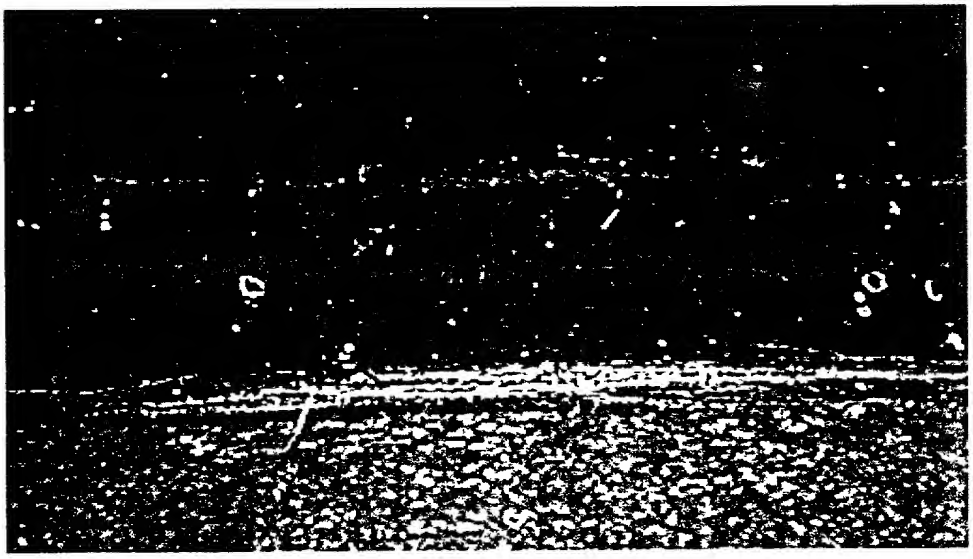


Figure 6B



~~Figure 7: Early Functional recovery~~

Figure 7A

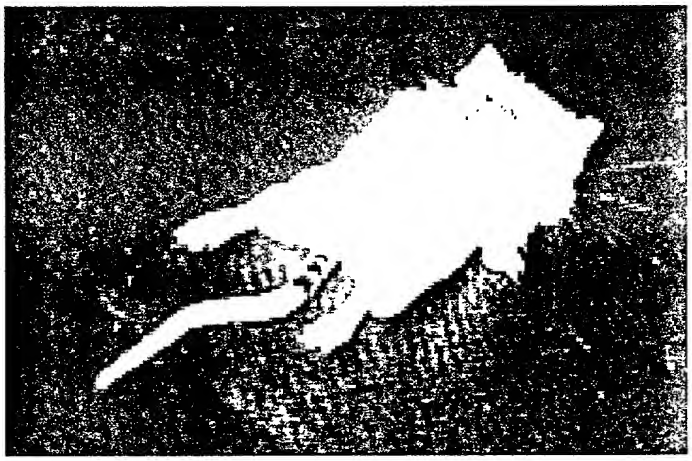


Figure 7b

Figure 7C

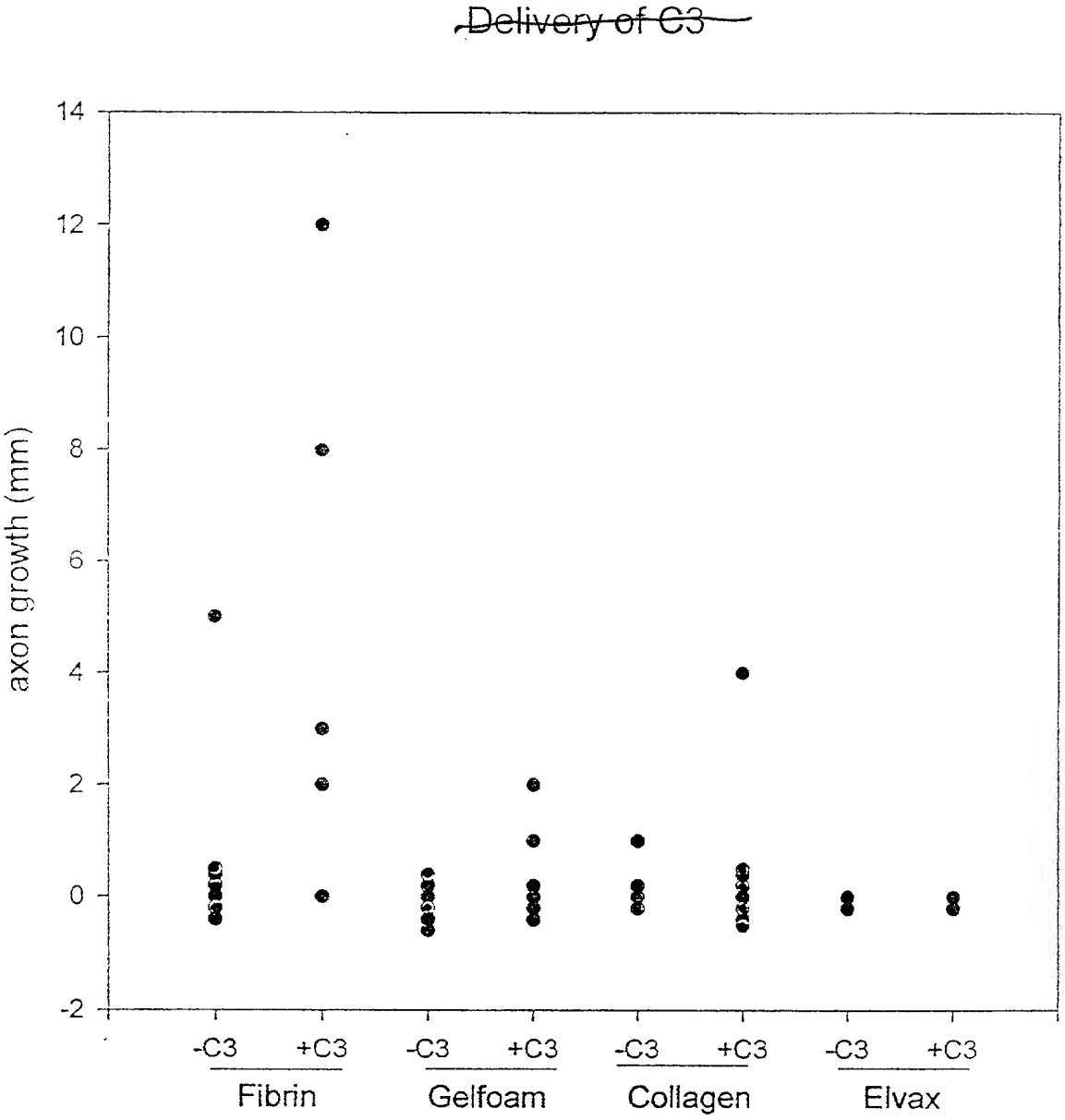
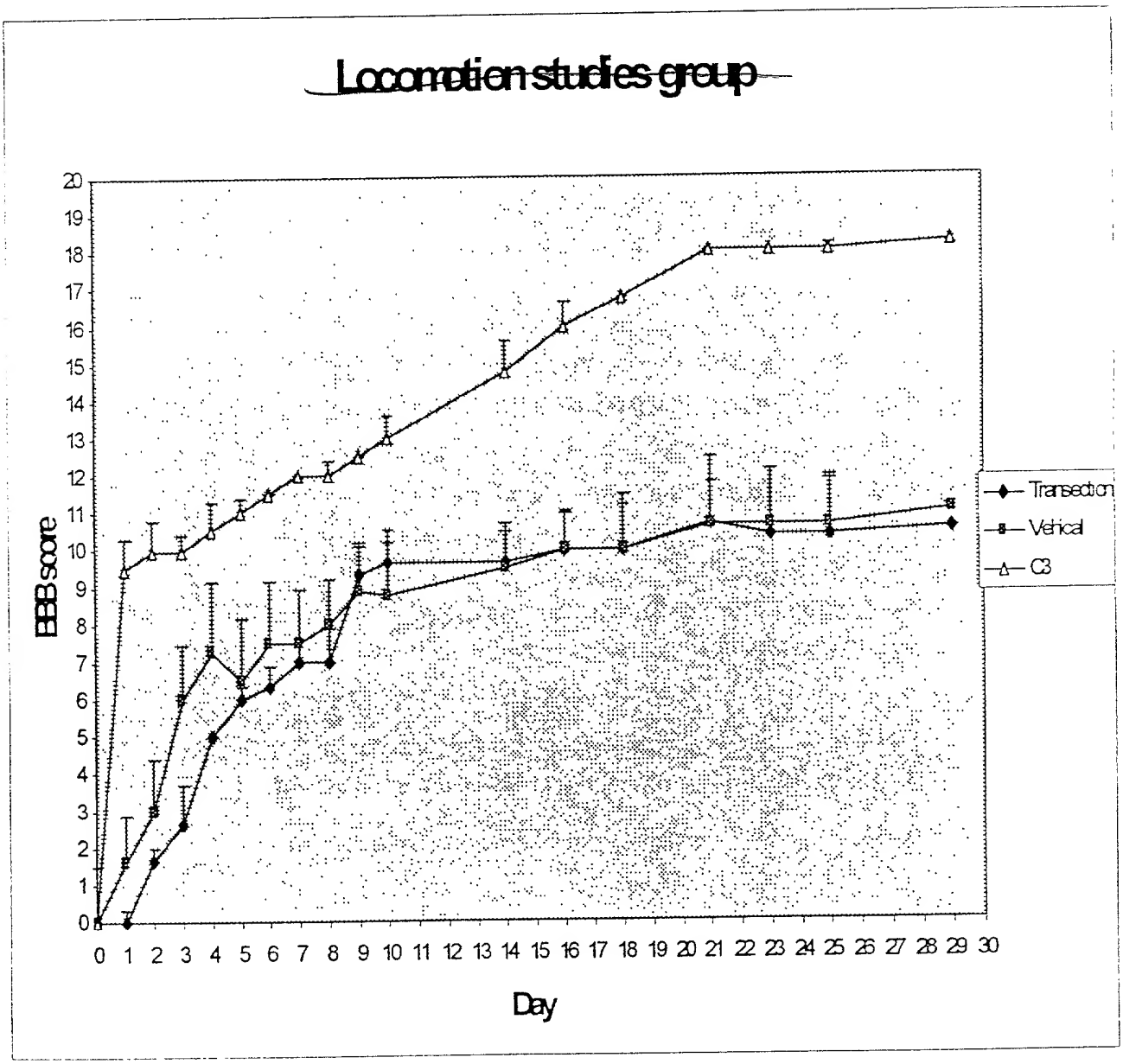

Delivery of C3
axon growth (mm)
14
12
10
8
6
4
2
0
-2
-C3
+C3
-C3
+C3
-C3
+C3
-C3
+C3
Fibrin
Gelfoam
Collagen
Elvax

Figure 8

BBB tests show recovery after C3 treatment



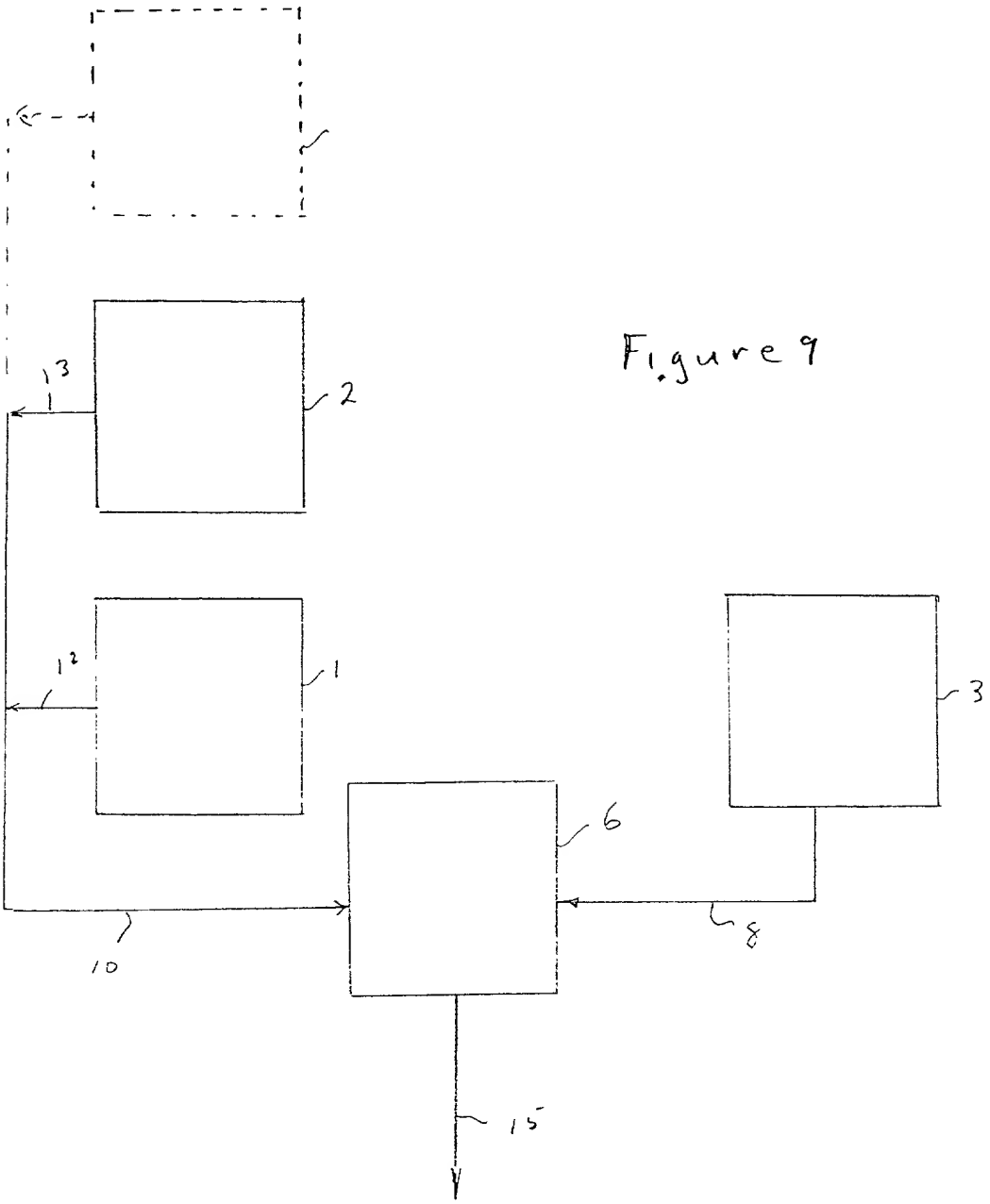


Figure 9